# The Female Reproductive Cycle in North Florida Kinosternon baurii (Testudines: Kinosternidae)

JOHN B. IVERSON<sup>1</sup>

Florida State Museum and Department of Zoology, University of Florida, Gainesville, Florida 32611

ABSTRACT.—Female striped mud turtles are generally active throughout the year, with maximum activity in March and October and minimum in February and July. Females mature at an age of 5 to 6 years and a plastron length of 70 to 75 mm.

Vitellogenesis is continuous from July through the following May. Ovulation and oviposition occur from at least September to June. At least three clutches are produced annually. Egg size averages  $28 \times 17$  mm and is not correlated with female size or clutch size. The mean length of laboratory incubation is 119 days and hatchlings average 19.2 mm PL. Clutch size ranges from one to five (usually two or three) and is positively correlated with female size. Each clutch comprises about 8 percent of total body weight.

## INTRODUCTION

Few reproductive studies exist for mud turtles (genus Kinosternon) other than Kinosternon subrubrum (Mahmoud and Klicka 1972; Gibbons 1975; Iverson, 1979) and Kinosternon flavescens (Mahmoud and Klicka 1972; Christiansen and Dunham 1972). Studies by Sexton (1960) of K. scorpioides and by Moll and Legler (1971) of K. leucostomum comprise the only other detailed studies. Most Kinosternon reproductive information is anecdotal.

The present knowledge of reproduction in K. baurii is based almost entirely on Einem's (1956) and Lardie's (1975) observations in central Florida. The purpose of this report is to provide more complete information on the female reproductive cycle of striped mud turtles, *Kinosternon* baurii from northern Florida. This study, with others now in progress by this author, should soon permit an analysis of reproductive strategies within the genus.

### MATERIALS AND METHODS

Female turtles were collected whenever possible in Alachua, Levy, and Marion counties in north Florida (usually within 50 km of Gainesville)

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<sup>&</sup>lt;sup>1</sup>Present address: Department of Biology and Joseph Moore Museum, Earlham College, Richmond, IN 47374.

from January 1972 through December 1976. Due to the proclivity of adults and hatchlings to terrestrial activity in north Florida (Carr 1952), this species is more frequently encountered on land than any other local turtle. Most turtles were collected as they attempted to cross roads. Many of these were dead on the road, but remained intact enough to provide suitable specimens for measurement and dissection. Turtles were also obtained by trapping, seining, and diving. In addition, specimens from north Florida in the Florida State Museum (University of Florida) collection were included in the samples.

Ovarian follicles, oviducal eggs, and corpora lutea were examined and measured in each specimen. All straight-line measurements were taken with dial calipers to the nearest 0.1 mm. Carapace length (CL) and plastron length (PL) were also recorded. Measurements from preserved turtles did not significantly differ from the data collected from fresh specimens and were included in the analysis.

Several clutches of shelled oviducal eggs were removed and incubated to hatching at 27° to 29°C. Data from eggs in natural nests were also recorded. Most turtles were deposited in the Florida State Museum, University of Florida, but a representative series was retained by the author. Means are followed by  $\pm$  one standard deviation. All measurements are in mm.

## SEASONAL ACTIVITY

Striped mud turtles were collected throughout the year, both on land and in water. Individuals may be found active on all but a few of the coldest winter days. This species thus exhibits the longest annual activity cycle of any previously studied kinosternid in the United States (Christiansen and Dunham 1972).

Annual terrestrial activity seems to be bimodal. Striped mud turtles are most frequently encountered (especially on land) in March when spring rains are filling the ponds and marshes, and in October as water levels are dropping. Few turtles were collected during late summer when water levels and temperatures are maximal, or in mid-winter (February) when temperatures are minimal. Wygoda (1976) found a similar annual activity pattern in K. *baurii* inhabiting seasonally flooded hardwood swamps in central Florida.

Since annual activity is bimodal, two (or more) epidermal scute annuli are often produced by an individual turtle during any one year. Winter annuli are apparently always produced and are usually more distinct than those produced in the summer. This variation in annuli production often makes aging turtles, based on scute annuli, difficult.

## AGE, GROWTH, AND SIZE AT MATURITY

Females possessing ovarian follicles exceeding 7 mm diameter (or oviducal eggs or corpora lutea) during the months of July through May were considered mature. The three smallest mature females had plastral lengths of 69.4, 70.2 and 72.7. The four largest females judged to be immature measured 66.6, 67.9, 74.8, and 79.6 PL. Maturity therefore occurs at sizes between 70 and 75 PL (75 to 80 CL). I believe the 79.6 PL immature was reproductively anomalous since nine other females with plastron lengths between 75 and 80 mm were mature. The smallest mature female dissected by Einem (1956) was 81.2 mm CL and the largest immature female was 71.1 mm CL.

Age at maturity was estimated by counts of clearly visible winter (or primary) abdominal scute annuli as described by Sexton (1959). The oldest immature females bore six clear primary annuli (74.8 and 79.6 PL); the youngest mature female had only five (73.1 PL), indicating the usual age at maturity to be five or six years.

Plastron lengths calculated from the abdominal scute annuli lengths of 19 juvenile and young adults in the manner of Ernst et al. (1973) indicated turtles in their first winter averaged 17.9 PL (Range = 14.4-20.6, N = 19). Those in their second winter averaged 32.7 PL (25.5-39.0, N = 19); in their third winter, 45.7 (34.3-56.8, N = 14); fourth, 57.8 (49.3-64.3, N = 11); fifth, 68.9 (62.8-76.9, N = 9); sixth, 74.3 (69.1-82.0, N = 8); and in their seventh, 75.1 (71.1-83.8, N = 7). If turtles are maturing at plastral lengths of 70-75, these data support the contention that maturity occurs during the fifth to sixth years of age.

Average size of 101 adult females previously examined from throughout Florida (Iverson 1978b) was 86.2 PL (91.6 CL) [52 males averaged 73.1 PL, 83.7 CL]. The largest female measured 105.1 PL and 114.7 CL. The PL-body weight (in gm) regression, based on six females (69.1-90.0 mm PL, and 64.5-143.1 gm), is Wt = 3.36 PL - 154.84 (r = 0.92; p < 0.01). From this regression the average female (86.2 PL) weighs 135.1 gm.

#### FEMALE REPRODUCTIVE CYCLE

The ovarian cycle is nearly continuous (Table 1), with only a short summer quiescent period (coincident with the summer reduction in activity). Ovulation occurs from late August or early September to early June. Based on excavated nests and the presence of oviducal eggs, females apparently nest from September through June. Females continue to yolk follicles to replace those ovulated during all but the last of this period. Follicular enlargement is curtailed only from late May through June. During the remainder of the year enlarged follicles (> 7 mm) are typically found in the female reproductive tract along with oviducal eggs and/or corpora lutea.

Ova were removed from oviducal eggs to determine their approximate size at ovulation. Twenty-two excised yolk masses averaged  $16.24\pm1.06$  (Range = 14.5-18.0) in diameter. Maximum diameter of an ovarian follicle was 17.5. Only four females had ovarian follicles exceeding 16 mm in diameter.

The ovaries of one dissected female (98.8 PL), collected 22 April 1972, were anomalous in that her ovaries bore no corpora lutea or follicles > 4 mm in diameter.

Of 50 females with oviducal eggs and corpora lutea, 16 exhibited evidence of transuterine migration of ova. Net migration was away from the tract with the larger ovary in 11 (68.8%) of the cases, and probably served to equalize reproductive tract volumes.

The length of time that eggs were retained in the oviducts is not known. No females with oviducal eggs had the corresponding corpora lutea in any state of regression; all appeared fresh (maximum corpora lutea diameter is 6-7 mm). A physiological mechanism such as that suggested by Moll and Legler (1971) may allow female K. baurii to retain their eggs until suitable nesting sites and conditions can be found, without possibility of subsequent ovulation.

Month	Sample Size	% with Enlarged Follicles	% with Oviducal Eggs	% with Corpora lutea					
					January	9	100	77.8	100ª
					February	2	100	50	50
March	25	100	56	92					
April	9	88.9	44.4	55.5ª					
	15	73.3	53.3	73.3					
June	7	57.1	28.6	42.9ª					
July	1	100	0	100					
August	4	100	25	25					
September	4	100	100	100					
October	9	100	88.9	100ª					
November	3	100	100	100					
December	7	100	100	100					

Table 1. Percentage of mature female Kinosternon baurii bearing enlarged follicles (> 7.0 mm), oviducal eggs, and corpora lutea for each month.

aincludes one female with two sets of corpora lutea.



Fig. 1. Relationship between clutch size and egg size (mean length for clutch in mm) in *Kinosternon baurii*. Each symbol represents at least one record. Least squares regression is  $y = -0.08 \times + 4.83$ ; r = -0.16, p > 0.5 (N = 49).



Fig. 2. Relationship between body size (PL in mm) and egg size (mean length for clutch in mm) in *Kinosternon baurii*. Each symbol represents at least one record. Least squares regression is  $y = 0.36 \times + 76.67$ ; r = 0.093, p > 0.05 (N = 38).



Fig. 3. Typical mid-incubation crack in egg of *Kinosternon baurii* after 103 days incubation. Oviducal egg was removed from female 26 September 1976, crack first appeared 74 days later. Egg hatched after 118 days incubation.  $\times$  marks top of egg.

Natural nests with eggs in various stages of incubation were found on the following dates: 8 March, 25 April, 7 May, and 20 May 1972; and 19 May (2), and 25 May (2) 1973. Eggs were found buried under 1-3 cm of sand (5 cases) or under moist leaf litter 1 cm or less in depth. Two captive turtles oviposited on 8 February and 17 February 1972, the former in sand and the latter in the water of its tank. Carr (1952) reported finding natural nests from April to June in sand and in piles of dead hyacinths. Captive nestings were observed on 7 March, 9 June, and 19 November by Lardie (1973), and on 4 June by Nicol (1970).

## EGGS, INCUBATION, AND HATCHING

The eggs of *Kinosternon baurii* have been described by Einem (1956) and present observations do not differ from his. Mean egg size for 114 eggs I examined was 27.55 (longest diameter; Range = 22.8-32.8) by 16.63 (shortest diameter; Range = 13.6-19.3). Shells of 2 eggs ( $28.6 \times 16.7$ ;  $28.6 \times 16.8$ ) were 0.23-0.28 mm thick at their ends (10 measurements) and 0.34-0.40 thick along the perimeter of their minor axes (10 measurements). Eight eggs (X length 26.6) averaged 4.45 gm each (3.22-5.26).



Fig. 4. Relationship between hatchling size (PL in mm) and egg length (mm) in Kinosternon baurii. Least squares regression is  $y = 0.611 \times + 1.846$ ; r = 0.813, p < 0.01 (N = 14).



Fig. 5. Relationship between clutch size (based on counts of corpora lutea) and body size in *Kinosternon baurii*. Each symbol represents at least one record. Least squares regression is  $y = 0.048 \times -1.630$ ; r = 0.442, p < 0.01 (N = 60).

Seventeen oviducal eggs from central Florida K. baurii measured by Einem (1956) averaged 28.0 (Range = 25.0-31.8) by 16.6 mm (15.8-17.2). Lardie (1975) recorded a single central Florida egg of  $28 \times 16$ , and Nicol (1970) measured six south Florida eggs which averaged 23.75 (21-27) by 14.0 (12.5-16). Egg length was not significantly correlated with clutch size (Figure 1) or female PL (Figure 2). No seasonal trends in egg size were identifiable.

Fourteen clutches of oviducal eggs were incubated. Three clutches were opened and preserved after 88, 109, and 114 days, respectively. The remainder were incubated to hatching. At least some of the eggs of all clutches exhibited mid-incubation cracking of the egg shell (Figure 3) as described for *Kinosternon baurii* by Einem (1956) and for *Sternotherus minor* by Iverson (1978a). Not all eggs in any one clutch developed these cracks. Crack formation occurred after an average incubation period of  $78.8 \pm 16.7$  days (Range = 58-113; N = 9 eggs).

Mean incubation time to hatching for 11 clutches was  $118.8 \pm 11.3$  days (Range = 97-143). Eggs from any single clutch hatched within 24 hours of each other, except for one clutch in which 9 days separated the hatching of the 2 eggs. Five eggs incubated by Einem (1956) hatched after 96 to 129 days. Nicol (1970) hatched 4 eggs from the same clutch after 91, 93, 102, and 107 days, respectively. Three eggs from a clutch laid 9 June 1969 were hatched by Lardie (1975) after 117-119 days. Clutches hatched in my laboratory in January (1), February (1), March (3), April (1), June (1), August (2), and September (1). The hatching process was as described by Einem (1956).

Twenty-eight captive hatchlings averaged 19.2 PL (Range = 15.45-22.0) and 22.5 CL (Range = 17.55-25.0). Plastron length of hatchlings was significantly positively correlated (r = 0.813) with egg size (Figure 4). Hatchling-sized turtles were collected in the field on 5 January, 27 January (8), 28 January (5), 4 March (4), 9 March (2), 7 August, 15 September, and 7 December. Most of these had incompletely or very recently closed umbilical openings. Average size of 13 of these neonates was 19.6 PL (Range = 16.55-22.0) and 22.4 CL (Range = 20.4-25.0). Hatchlings from three eggs incubated by Lardie (1975) each measured 19 PL (22 CL); five hatched by Einem (1954) measured 18.1-20.3 PL (20.5-25.0 CL); and four hatched by Nicol (1970) averaged 17.5 CL (15.0-18.5).

## CLUTCH SIZE

Clutch size in *Kinosternon baurii* ranged from one to five, two or three being the usual complement. Mean clutch size was insignificantly different whether estimated by counts of enlarged follicles over 10 mm diameter  $(2.69\pm0.85; N = 48)$ , oviducal eggs  $(2.60\pm0.96; N = 50)$ , or corpora lutea ( $2.50\pm0.87$ ; N = 82). Twelve clutches examined by Einem (1956) and Lardie (1975) averaged 2.33 (Range = 1-3). Nicol's (1970) record of a 6-egg clutch probably represents the maximum for the species. As Einem's (1956) data suggest, clutch size is positively correlated (p < 0.01) with plastron length and increases an average of one egg for each 20.8 mm increase in PL (Figure 5). No seasonal trends in clutch size were identifiable.

### ANNUAL REPRODUCTIVE POTENTIAL

The ovaries of four females bore two distinct sets of corpora lutea. Clutch sizes of these turtles were 3 (larger set of corpora lutea) and 2 (6 October; 95.4 PL), 2 and 2 (5 January; 101.3 PL), 1 and 1 (20 April; 77.8 PL), and 5 and 4 (20 June; 95.4 PL). All but the last of these also had a set of pre-ovulatory follicles > 10 mm diameter. The ovaries of the first female suggest that following the first clutch of the reproductive season (late August to early September ?), she might possibly nest again in October. If her indicated inter-nest period of about 2 months were maintained, 6 clutches could be produced annually. This relatively long internest interval presumably allows for nearly complete luteal regression between ovulations and may explain why only 4 of 84 females had more than one set of identifiable corpora lutea.

Mature females certainly produce at least three clutches each year. Einem (1956) also suspected that three clutches might be produced annually, and Lardie (1975) reported a captive central Florida female (115 mm CL) which produced three clutches of three eggs each on 7 March, 9 June, and 16 November 1969. If each clutch averages 2.5, average annual reproductive potential is at least 7.5. Field studies will be necessary to determine the actual number of annual clutches.

Reproductive effort per clutch was estimated by the ratio of mean clutch weight (mean egg weight  $\times$  mean clutch size) to mean total female weight. Clutches in *Kinosternon baurii* average 8.23 per cent (4.45 $\times$ 2.50 $\times$ 100/135.1) of female weight. Unfortunately, similar estimates are available for no other kinosternid turtle.

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#### John B. Iverson

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